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Pollination of *Plectranthus* spp. (Lamiaceae) with sigmoid flowers in southern Africa

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Abstract

Within the South African *Plectranthus* species two specialized lines of corolla adaptations have evolved. Long-proboscid flies (Nemestrinidae) appear to have driven the development of *Plectranthus* species with long corolla tubes that are limited to the sub-continent. *Plectranthus* s.l. (including *Coleus*) species with sigmoid corollas are far more widespread and evidence presented here supports the hypothesis that this floral type has evolved as a response to melittophily. Thirty percent of southern African *Plectranthus* species have corolla tubes that are bent to some degree. Pollination of the following four labiate species with sigmoid corollas was studied in detail: *Plectranthus petiolaris*, *P. laxiflorus*, *P. calycinus* and *Pycnostachys urticifolia*. The pollination of three other species was investigated to a lesser degree: *Plectranthus spicatus*, *P. rehmannii* and *Aeollanthus parvifolius*. Bee pollination is confirmed for *P. laxiflorus* and *Py. urticifolia* and is recorded here for the first time in *P. petiolaris* and *P. calycinus*. A new group of floral visitors comprising nemestrinid flies of the genus *Prosoeca* with moderately long proboscids is recorded for *P. laxiflorus* and *P. calycinus*, where the corolla tube shape allows visits by medium-proboscid floral visitors. The sigmoid corolla shape limits the type and size of insects that can access nectar and act as pollinators. Explanations for the existence and function of the sigmoid corolla shape are suggested.

Keywords: Apidae; Anthophorinae; Lamiaceae; Nemestrinidae; *Plectranthus*; Pollination; Sigmoid corolla; Southern Africa

1. Introduction

Plectranthus (Lamiaceae), a member of subfamily Nepetoideae, tribe Ocimeae, subtribe Plectranthinae (Paton et al., 2004), comprises ±300 species that occur in the tropical and warm regions of the Old World (Retief, 2000). It is the largest genus of the Lamiaceae in southern Africa, represented by ca. 53 species (Codd, 1975, 1985; Van Jaarsveld and Edwards, 1991, 1997; Van Jaarsveld and Hankey, 1997; Edwards et al., 2000; Van Jaarsveld and Van Wyk, 2004; Edwards, 2005; Winter and Van Jaarsveld, 2005). Recent evidence (Paton et al., 2004) suggests that the genus is paraphyletic as currently circumscribed.

In an overview of pollination biology in the Lamiaceae, Huck (1992) commented on the large gaps that exist in our knowledge and recommended that pollination studies focus on species *in situ*. In view of the large size of the genus we identified it as an ideal group for pollination studies. The pollination of long-tubed *Plectranthus* species by long-proboscid flies was first reported by Potgieter et al. (1999), along with the pollination of a number of medium- and short-tubed species of the genus by bees and flies with medium- to short proboscids. The discussion surrounding long-tubed species of *Plectranthus* was extended to the rest of the Lamiaceae in southern Africa by relating the distribution of long-tubed Lamiaceae to the biogeography of long-proboscid flies (Potgieter and Edwards, 2001). Pollination of Lamiaceae with corollas of intermediate length, by pollinators with intermediate length proboscids, may have predisposed the group to

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pollination by long-proboscid flies, with subsequent extension of corolla tubes. Elongation of corolla tubes leads to increased protection of nectar resources, which leads to increased pollinator fidelity (Potgieter and Edwards, 2001). Publication of the *Stenobasipteron wiedemanni* (Diptera, Nemestrinidae) Pollination Guild (Potgieter and Edwards, 2005) established the multi-family plant guild to which long-tubed species of *Plectranthus* (with tube lengths of 20–33 mm) belong.

Both the specialized long-proboscid fly pollinated and more generalized shorter-proboscid fly and bee pollination guilds that have been studied within southern African *Plectranthus* (Potgieter et al., 1999; Potgieter and Edwards, 2001, 2005) involve species with relatively straight corolla tubes. A new pollination guild is described here for species with sigmoid-shaped corollas.

Initial phylogenetic work by Paton et al. (2004), supplemented by Lukhoba et al. (2006), creates a framework for phylogenetic interpretation of *Plectranthus* and its allies. Subsequent work shows that there are three main clades in the genus *Plectranthus*, but the relationships between them are unresolved (A. Paton, pers. comm.). They are: 1) a sigmoid '*Coleus*' clade, including *P. rehmannii* Gürke and *P. calycinus* Benth., possibly *P. spicatus* E. Mey. ex Benth,. and *Pycnostachys urticifolia* Hook.; 2) a sigmoid *Plectranthus* clade, including *P. petiolaris* E.Mey. ex Benth. and *P. laxiflorus* Benth.; and 3) a straight *Plectranthus* clade. The genus *Aeollanthus* Mart. ex K.Spreng is placed near the base of the Plectranthinae (A. Paton, pers. comm.). The definition of sigmoid corollas by Codd (1985) included corollas with varying degrees of geniculation of the tube, and for the purpose of this paper the term 'sigmoid' is used in a loose sense. Within southern African *Plectranthus* 30% of species have evolved short to medium sigmoid corollas similar to those in *Solenostemon* Thonn., *Aeollanthus* and *Pycnostachys* Hook. These three genera all fall within the broader generic circumscription of *Plectranthus* proposed by Paton et al. (2004).

The bulk of this paper deals with four species that were studied in detail: *P. petiolaris* and *P. laxiflorus*, with sigmoid tubes bent to a similar degree; *P. calycinus* Benth. [=*Rabdosiella calycina* (Benth.) Codd], with weakly bent corollas, and *Pycnostachys urticifolia* Hook., with distinctly bent corollas. Three other species were studied in less detail, but are recorded and discussed since they fall within the same syndrome: *P. spicatus* (with distinctly bent corollas), *P. rehmannii* (with weakly bent corollas), and *Aeollanthus parvifolius* Benth. (with curved corolla tubes).

During the course of the study it was noted that a site in Pietermaritzburg with extensive stands of *P. laxiflorus* showed a temporal separation of pollinator classes during each flowering season. At first only bee pollinators were present, but nemestrinid flies emerged *en masse* at the end of March/early April each year, with bees and flies actively visiting flowers for the rest of the season. An investigation on the effectiveness of the two pollinator types was included in the study.

2. Literature on sigmoid Lamiaceae

In a discussion on corolla adaptations in the Lamiaceae, Meeuse (1992) noted two important aspects: the size of specialized corollas and the resistance offered to insect visitors attempting to access nectar. The upper corolla lip of *Plectranthus* is erect and does not hamper access, whereas the lower lip allows for easy landing, thus some kind of barrier in the tube would function as a selective device (Meeuse, 1992). Van der Pijl (1972) considered why some *Plectranthus* species, as a transition to *Coleus*, should have 'geniculate' corolla tubes. One suggestion was that the bend may be a mechanical necessity for a horizontal flag-type blossom (with an upright upper limb) to combine with the long vertical tube that already exists in this group (Van der Pijl, 1972). Another is that sigmoid corollas provide an effective shift from butterfly to bee pollination without major changes in tube dimensions (Van der Pijl, 1972), possibly since the sigmoid shape may allow bees better access to nectar than in a long-tubed, straight corolla.

There are no published records on the pollination of *P. petiolaris* and only one for *P. laxiflorus* (Scott Elliot, 1891). In *P. calycinus* the sides of the lower lip are bent upwards, giving it a boat-like appearance, enclosing the stamens and style. As an insect visitor forces the lower lip down the stamens are exposed, dusting pollen onto the insect. In both *P. calycinus* and *P. laxiflorus* this action requires considerable force from the insect (Scott Elliot, 1891) and the corolla shape of *P. petiolaris* suggests that a similar system operates in this species. Van der Pijl (1972) mentioned the bee-blossoms of *P. laxiflorus* and tried to explain their sigmoid corolla shape. It was first suggested that the bend in the corolla tube may have functioned in excluding bee visitors while fitting a lepidopteran proboscis, yet the fused filaments and hinged carina suggests bee visitors, or a regression to melittophily (bee pollination). Vogel (1954) listed *Plectranthus* under melittophily and sphingophily (moth pollination), while *Pycnostachys* was placed under melittophily. Percival (1965) discussed how bees with abdominal brushes (the Dasygastrae) exploit sternotrobic flowers, such as *Py. urticifolia*, by collecting pollen ventrally on the abdomen. She mentioned that the open corolla mouth of the labiates did not hinder bees from probing, but that visitors were stratified according to tube length.

Stirton (1977) described the insect visitors to cultivated plants of the South African species *Plectranthus neochilus* Schltr. The corolla of this species has a narrow tube (15–18 mm long) that ascends, then bends knee-like and expands about the middle (Codd, 1985). Although the corolla tube is not bent to the same extent as found in *P. petiolaris* and *P. laxiflorus*, *P. neochilus* makes for an interesting comparison. Stirton (1977) found five species of *Megachile*, three *Xylocopa* species, one species of *Anthophora* (now genus *Amegilla*) and *Apis mellifera* (all Hymenoptera, Apidae) to be effective pollinators. The bees landed on the boat-shaped lower lip, depressed it and exposed the stigma and stamens which transferred pollen ventrally onto the insects (Stirton, 1977). This study also listed unidentified bombyliids (Diptera: Bombyliidae), one syrphid species (Diptera: Syrphidae) and a sphingid moth, *Macroglossum trochilus* (Lepidoptera: Sphingidae), as ineffective visitors.

Paton et al. (2004) noted that the sigmoid tubes of the *Coleus* clade and the sigmoid *Plectranthus* clade (*P. laxiflorus* and *P. petiolaris*) always combine with a horizontal lower (anterior) corolla lobe, a combination which they believed would favour landing insects with flexible proboscids.

3. Materials and methods

All seven plant species were studied in KwaZulu-Natal (KZN), with some work done in the Eastern Cape Province of South Africa (Fig. 1a). Field work was conducted in various localities from 1995 to 2009 (see Appendix 1 for study site, year of study and voucher details). Plant species were identified using Codd (1985), as well as the texts listed in the introduction, for species described subsequent to Codd's (1985) revision.

Plant distributions were compiled from flora accounts (Codd, 1985), herbarium records (NU, NH and PRE) and field observations. Pollinator observations were made across a number

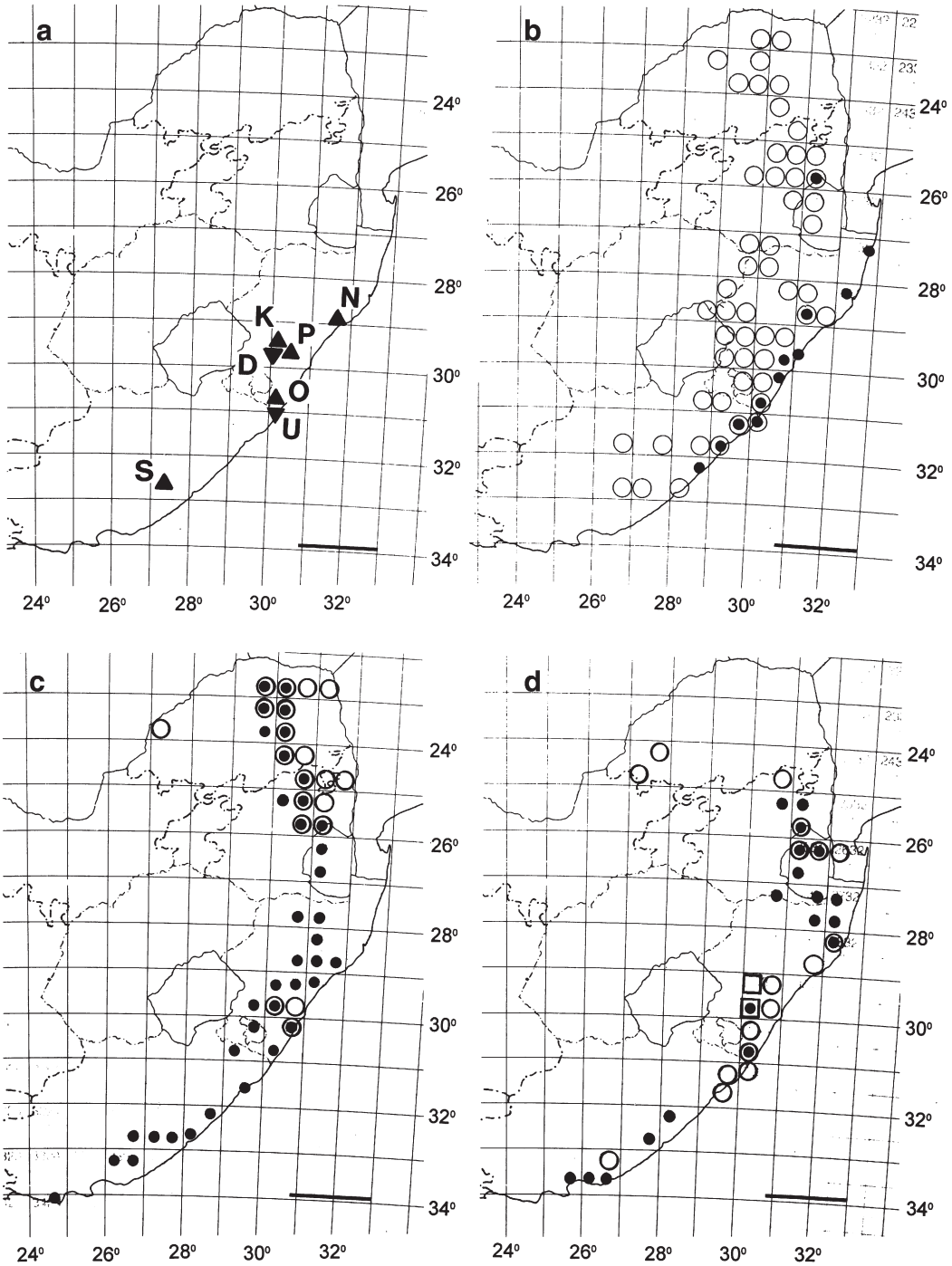

Fig. 1. Distribution maps for study species and field study sites: (a) Study sites in eastern South Africa; (b) distribution of *P. petiolaris* (solid circles) and *P. calycinus* (open circles); (c) distribution of *P. laxiflorus* (solid circles) and *Py. urticifolia* (open circles); (d) distribution of *P. spicatus* (closed circles), *A. parvifolius* (open circles) and *P. rehmanii* (open squares). S = Stutterheim–Kologha Forest, U = Umtamvuna N.R., O = Oribi Gorge N.R., D = Dargle, P = Pietermaritzburg, K = Karkloof–Leopards' Bush N.R., N = Ngoye Forest. Bar: 200 km.

of flowering seasons, between 7.00 am and 6.00 pm. Insects that visited study flowers were netted and asphyxiated in separate glass pill vials to prevent pollen transfer between specimens. Vials were prepared by compressing absorbent paper into the base of each vial and saturating it with ethyl-acetate. Bees and flies were pinned with proboscids extended forward.

Insect voucher specimens are lodged with the Natal Museum in Pietermaritzburg (Diptera) and with the Biosystematics Division, Plant Protection Research Institute, Pretoria (Hymenoptera). Hymenoptera were classified according to Brothers (1999). Plant vouchers are housed at the Bews Herbarium, University of KwaZulu-Natal (NU) (Appendix 1).

Areas of pollen deposition on insects were determined using a dissecting microscope. Initially pollen samples were removed with double-sided tape for Scanning Electron Microscopy (SEM). Stubs were coated with gold-palladium and examined under a Hitachi S570 scanning electron microscope at an accelerating voltage of 10 kV. Pollen grains of *Plectranthus* and *Pycnostachys* species are morphologically similar, with 6-colpate grains and reticulate exine patterns, and can be distinguished from pollen grains of other plant families.

Percentages of *Plectranthus* to total pollen loads were estimated. For *Pycnostachys*, representative voucher insects were killed and examined, but subsequent specimens were caught and cooled to allow handling. Tiny cubes of Fuchsin gel were used to remove pollen from different areas on the insect body, placed on separate slides and gently heated to make semi-permanent mounts. Insects were marked with tiny drops of white correction fluid on the thorax to avoid re-examination if captured subsequently. Slides were examined with a compound light microscope to determine the percentage *Pycnostachys* pollen present. The Fuchsin gel technique was followed in later years.

Proboscis length measurements were made from the tip of the proboscis to the point where the proboscis attaches to the head of the insect. In the case of bees this measurement was divided into the length of the solid base of the proboscis (galea) including the clypeus, and the protruding flexible part (glossa).

Corolla tube lengths were measured using a fine piece of wire bent in the shape of the sigmoid corolla, extending from the corolla base to the point where the upper and lower corolla lips diverge. The wire was then straightened and measured. Filament and style measurements include the entire, functional length of the corolla, although filaments attach to the corolla for some distance. Measurements were made from fresh, preserved and pressed plant specimens.

Nectar levels were recorded in *P. petiolaris* by comparing the height of the nectar column from the base of the corolla, with the distance from the base to the bend in the corolla. This was measured in unvisited flowers, early in the morning (at 9.00 am) and again at 2.00 pm (after many bee visits on a sunny day). The ratio of nectar column to 'base-to-bend' distance was compared between the morning and the afternoon.

To gauge the relative efficiency of apinid bees and nemestrinid flies in pollinating *P. laxiflorus* at Ferncliff Nature Reserve (NR), Pietermaritzburg, fruit set was recorded for two intervals: before (bees only) and after fly emergence (bees and flies). As soon as flies emerged in 2003, the position of the most recently opened verticil of flowers was marked on each of 16 inflorescences. At the end of the flowering season the resulting infructescences were collected. To ensure a suitable time lapse between observed and actual fly emergence, fruit set was only recorded from verticils three rows below the mark on each inflorescence (to represent 'bee only' visitation) and then from the verticil above the mark (to represent 'bee and fly' visitation). Fertilisation rate was calculated by comparing actual fruit set (no. of swollen calyces) with potential fruit set (no. of pedicels with and without calyces, representing no. of flowers).

By accessing unpublished data on other straight-tubed species of *Plectranthus*, combined with data on sigmoid species, twenty species were first grouped by corolla shape and length and then graded by reliance on shaded (forest) through to sunny habitat. The proportion of visits received by effective fly pollinators versus bee pollinators was estimated for each species, using pollinator observation and voucher data. Flies included the families Nemestrinidae, Tabanidae and Acroceridae; bees included the sub-families Apinae and Megachilinae of the family Apidae.

4. Results

The study species are more or less widely distributed along the eastern seaboard of southern Africa, with more than one species often co-occurring and one species (*P. rehmannii*) endemic to the KZN Midlands (Fig. 1b–d; Appendix 1). The habit, floral characteristics and habitat varies between species, but all flower in late summer to autumn (see Table 1).

Two floral subtypes were represented (see Table 2): flowers up to 11 mm long, with narrow corolla bases: *P. laxiflorus* (Figs. 2, 7c), *P. petiolaris* (Figs. 3, 7a) and *Py. urticifolia* (Figs. 5, 7b); and smaller flowers less than 7 mm long, with saccate bases: *P. calycinus* (Fig. 4). In *Py. urticifolia* the filaments are fused for a few millimetres beyond the corolla tube, enclosing the style in a rigid sheath that provides mechanical support once the bent filaments and style elongate and straighten after anthesis (Fig. 7b).

Sigmoid *Plectranthus* flowers have tubular corollas and are zygomorphic, with the stigma and four anthers enclosed by the lower lip (Fig. 7a–d). Flowers are protandrous, with anther dehiscence in the male phase followed by an extension of the style, bringing the stigma upwards into a position previously occupied by the anthers. The style is smooth and generally pin-like in appearance, with closely appressed bifid stigmatic lobes which open to reveal inner receptive surfaces during the female phase (Fig. 7c). The flowers are herkogamous, with the filaments and anthers dropping down into the boat-shaped lower lip of the flower during the female phase. In some of the non-sigmoid *Plectranthus* species with long filaments (e.g. *P. ecklonii* Benth.) the anthers and filaments curl away sideways after dehiscence (Potgieter et al., 1999).

The upright upper limbs of the bilabiate flowers are held vertically in all studied species and function in advertising nectar, which is secreted by a nectariferous disc around the ovary at the base of the corolla; nectar guides, when present, are most often located on the inner or adaxial surface of the upper corolla limb.

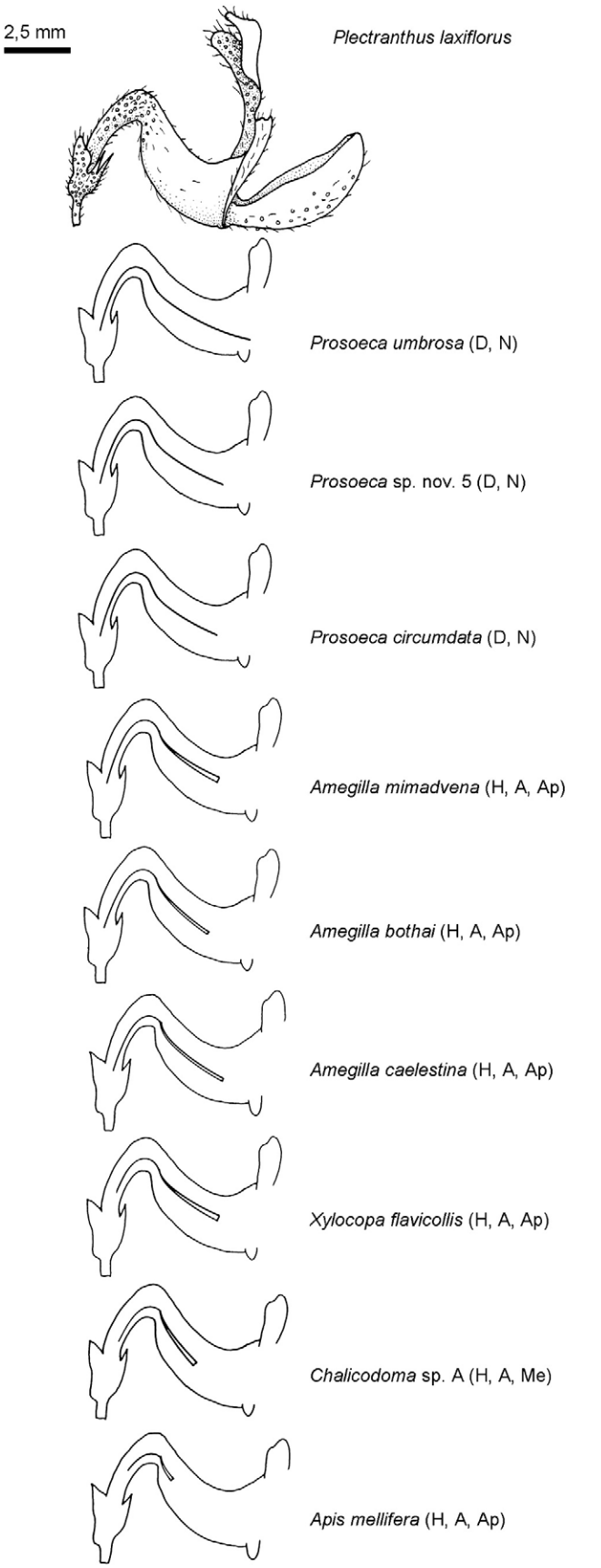

Fig. 2. Comparisons of floral tube shape and length with proboscids of insect visitors to *Plectranthus laxiflorus*. In bees the single lines show the proximal flexible part of a proboscis (glossa) that extends along the whole length; double lines show the rigid basal part (clypeus and galea). D = Diptera, N = Nemestrinidae, H = Hymenoptera, A = Apidae, Ap = Apinae, Me = Megachilinae. Bar: 2.5 mm.

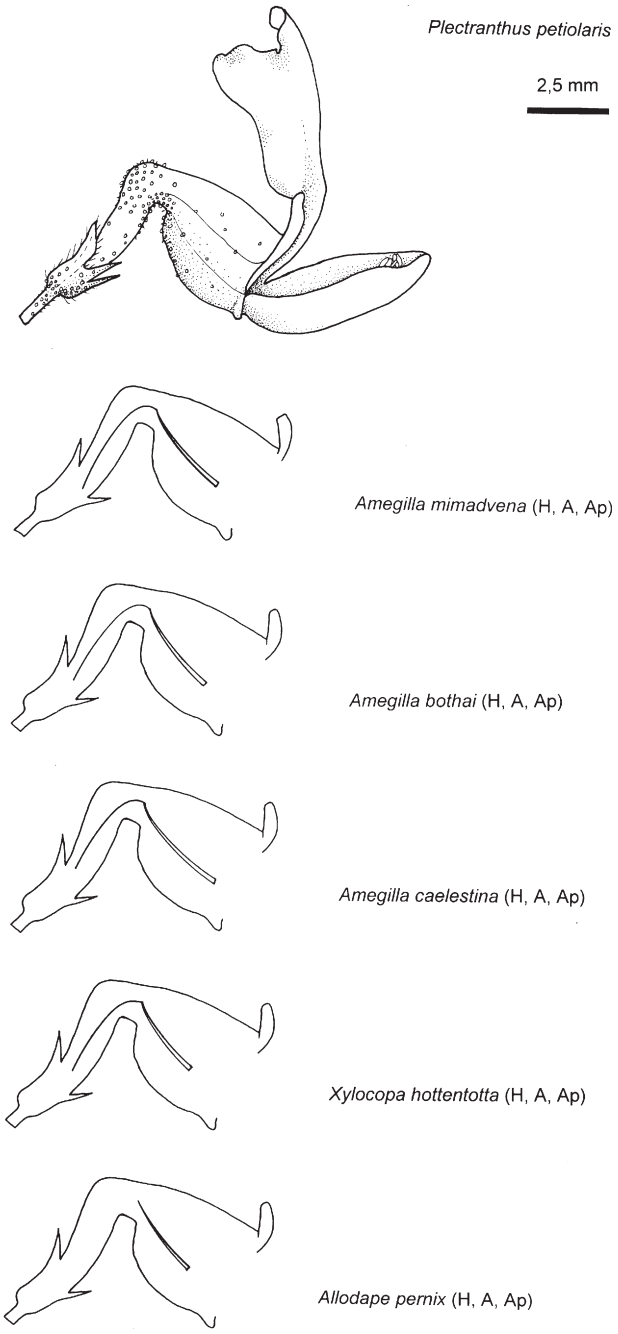

Fig. 3. Comparisons of floral tube shape and length with proboscids of insect visitors to *Plectranthus petiolaris*. Single lines show flexible parts of proboscis, double lines show solid parts. H = Hymenoptera, A = Apidae, Ap = Apinae. Bar: 2.5 mm.

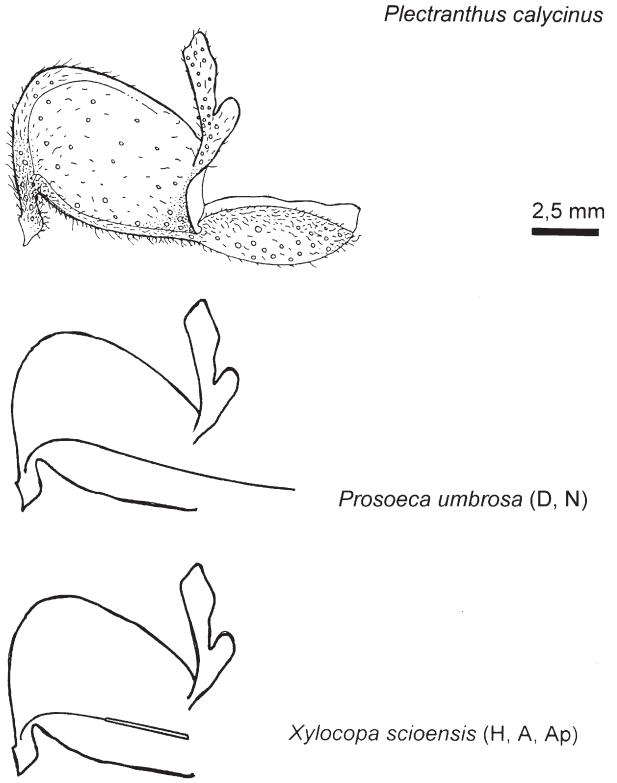

Fig. 4. Comparisons of floral tube shape and length with proboscids of insect visitors to *Plectranthus calycinus*. Single lines show flexible parts of proboscis, double lines show solid parts. D = Diptera, N = Nemestrinidae, H = Hymenoptera, A = Apidae, Ap = Apinae. Bar: 2.5 mm.

Table 1
Floral, plant and habitat characteristics of the four main study species.

Species	Habit	Habitat	Flowering time	Flower colour
P. petiolaris	Branched herb	Scree below cliffs covered by scarp forest; forest margins	December to April	Deep purple in northern KZN and Umtamvuna NR; pink at Oribi Gorge NR
P. laxiflorus	Freely branching soft shrub or herb, up to 1.5 m tall	Forest margins; damp open vegetation	Mid-February to April (sporadic in October and November)	White, with 4–5 thin purple linear nectar guides on flag-like upper lip
P. calycinus	Erect, branched shrub with slightly woody annual stems from woody rootstocks, stems up to 1.5 m tall	Grasslands	January to May (sporadically to July)	Creamy-white, tinged with mauve on edges of the corolla limbs
Py. urticifolia	Erect herb or shrub with a woody base, 1–2.5 m tall	Moist areas, such as forest margins and grassy stream banks	April to June	Deep blue

Nectar levels are generally confined to below the bend of the corolla. The base of the corolla tube is held at a near vertical or oblique angle upwards before the tube bends downwards; this presumably acts gravitationally to retain nectar. The nectar level fluctuation that was measured in *P. petiolaris* (by comparing the height of the nectar column from the base of the flower with the 'base-to-bend' corolla tube length), showed that unvisited flowers had an average nectar to tube bend ratio of 0.88 (SD 1.00, $n=27$) and visited flowers had an average ratio of 0.41 (SD 1.26, $n=31$). Thus in the morning newly secreted nectar was pushed up closer to the bend in the corolla tube, but later in the day it became more difficult for insects with shorter proboscids to extract nectar, as the nectar level dropped due to repeated insect visitation.

Flower colour varies from predominantly white (*P. laxiflorus*, *P. calycinus*) and creamy-white (*P. rehmannii*), to pale pink (*Aeollanthus parvifolius*), pink (*P. petiolaris* Oribi Gorge), deep purple (*P. petiolaris* Umtamvuna, *P. spicatus*) and deep blue (*Py. urticifolia*) (Table 1). In sigmoid species the darker coloured species tend to be pollinated by bees only, while the paler, whitish species appear to favour bee and fly pollinators. This pattern does not, however, extend to the rest of the genus when data from 20 species with a range of corolla shape is compared (C. Potgieter, unpubl. data).

Insect identifications were obtained for most bee species, excepting the megachilinid and halictinid bees, where generic identities are provided (Table 3; Appendix 2). Few KwaZulu-Natal specimens were included in the revision of nemestrinid flies done by Bezzi (1924), but subsequent work done by Barraclough (2006) allowed him to identify most *Prosoeca* (Diptera, Nemestrinidae) specimens to species-level for this study. Some species are new collections awaiting description (D. Barraclough, pers. com.).

The main daily period of activity of insect visitors was between 9.00 am and 4.00 pm. Apinid bees of the genera *Amegilla* and *Xylocopa* are the main pollinators of *Plectranthus petiolaris* (Fig. 3; Table 4). Bees of the genera *Amegilla* and *Xylocopa*, and pollen-collecting megachilinid bees of the genera *Megachile* and *Chalicodoma*, pollinate *Py. urticifolia* (Fig. 5; Table 4). *Plectranthus laxiflorus* is pollinated by species of *Chalicodoma* (Megachilinae), *Amegilla* and *Xylocopa* (both Apinae), as well as nemestrinid flies of the genus *Prosoeca*, of

Table 2
Mean floral measurements (in mm) for the four main studied sigmoid species: tube length measured from base of corolla to junction with lower lip; filament and style measurements include the full length of tube; *SD* standard deviation (in brackets after mean); *n* sample size.

Species (*n*)	Corolla tube (SD)	Style (SD)	Upper filament (SD)	Lower filament (SD)
P. petiolaris (22)	10.9 (0.7)	15.1 (4.2)	14.3 (0.6)	15.3 (2.3)
P. laxiflorus (18)	10.5 (0.9)	16.9 (1.8)	15.9 (1.3)	17.4 (1.4)
P. calycinus (29)	6.6 (0.6)	11.7 (1.1)	10.5 (0.8)	11.6 (0.9)
Py. urticifolia (26)	11.0 (0.7)	19.3 (1.0)	17.2 (1.1)	18.6 (1.0)

Table 3
Proboscis (and components of proboscis) length measurements of bee and fly visitors to all of the seven studied species.

Visitor	Average proboscis length (mm)			*N*
	Solid base (SD)	Flexible tip (SD)	Total length (SD)	
Prosoeca umbrosa			10.5 (0.8)	10
Prosoeca circumdata			9	1
Prosoeca sp. nov. 5			10	1
Amegilla mimadvena	5.1 (0.4)	3.9 (0.4)	9 (0.3)	6
Amegilla caelestina	4.3 (0.3)	4.2 (0.5)	8.5 (0.5)	12
Amegilla bothai	4.8 (0.3)	3.7 (0.3)	8.6 (0.4)	11
Amegilla fallax	4	3	7	1
Xylocopa hottentotta	4.2 (0.2)	3.3 (0.6)	7.5 (0.5)	3
Xylocopa scioensis	3.5	3.0	6.5	1
Xylocopa flavorufa	3.5	3.0	6.5	1
Xylocopa flavicollis	3.3 (0.4)	3.3 (0.4)	6.5 (0.7)	2
Thyreus sp.	4.3 (0.4)	2.0 (0)	6.3 (0.4)	2
Chalicodoma sp. A	3.0 (0)	2.8 (0.4)	5.8 (0.4)	2
Chalicodoma sp. B	2.0 (0.4)	2.8 (0.3)	4.8 (0.5)	4
Apis mellifera			3.3 (0.3)	5
Allodape pernix			3.2 (0.5)	8
Megachile sp. A			3	1
Megachile sp. B			3	1
Pseudoanthidium truncatum			2.8 (0.8)	2

SD standard deviation (in brackets after measurement); *n* sample size.

Table 4
Pollen placement and pollen loads (% *Plectranthus*/*Pycnostachys* pollen) on effective insect visitors of the four main study species.

Labiate species: with insect visitor	Pollen placement (ventrally)	Pollen load (%)
P. petiolaris		
Amegilla mimadvena	Thorax, abdomen.	25–95–100
	Scopae.	50
Amegilla caelestina	Thorax, abdomen.	100
Amegilla bothai	Thorax: between leg bases.	50–75
Xylocopa hottentotta	Thorax: between leg bases.	50
P. laxiflorus		
Amegilla mimadvena	Base of proboscis, head/thorax junction, abdomen, hind legs, scopae.	100
	Thorax: between leg bases.	95
Amegilla caelestina	Thorax, abdomen.	100
Amegilla bothai	Thorax, abdomen, scopae.	75–90
Prosoeca circumdata	Base of proboscis, head/thorax junction, thorax: leg bases, abdomen.	100
Prosoeca umbrosa	Head/thorax junction, thorax: leg bases	100
Prosoeca sp. nov. 5	Head/thorax junction, thorax, abdomen	100
Chalicodoma sp. A	Thorax, abdomen.	60–10
Xylocopa flavicollis	Head/thorax junction, thorax: between leg bases.	90
	Scopae.	75
P. calycinus		
Prosoeca umbrosa	Base of head, head/thorax junction, thorax, abdomen.	100
Py. urticifolia		
Amegilla mimadvena	Thorax, abdomen.	90
Apis mellifera	Thorax, abdomen, scopae.	100
Xylocopa scioensis	Head/thorax junction, base of proboscis.	100
	Thorax, abdomen.	95
Xylocopa flavorufa	Proboscis, head.	100
	Thorax, abdomen, hind legs.	75–100
	Thorax: bases of hind legs.	90
Megachile sp. A	Abdomen, hind legs	95–100
Megachile sp. B	Proboscis, base of head, thorax, thorax: leg bases, abdomen, hind legs	100
Chalicodoma sp. B	Abdomen	75 (few grains)

which *P. umbrosa* is the most abundant (Fig. 2; Table 4). *Prosoeca umbrosa* also pollinates *P. calycinus* at the Dargle, but is replaced by the apinid bee *Xylocopa scioensis* at Umtamvuna NR (Fig. 4; Table 4).

In all cases these insects were the most abundant visitors to the plants under study and all populations under study showed high levels of fruit set, as evidenced by the retention of swollen calyces. Under greenhouse conditions, where bees and flies are excluded, fruit set in the study species was found to be negligible, confirming that insects are necessary for pollination. Hand-pollination experiments done in a few non-sigmoid species of *Plectranthus* indicate that most species can set fruit from geitonogamous pollen transfer, but a few species do not (C. Potgieter, unpubl. data). Observations made on a single clone of the sigmoid *P. petiolaris*, grown in a garden, show that geitonogamous fruit set is possible.

In the four species that were studied in greater detail the edges of the lower corolla lip are folded inwards to partially conceal the anthers of unvisited flowers until a suitable insect visits the flower. Bees and flies with flexible proboscids of appropriate length can access nectar at the base of the corolla and, in the case of bees, the lengths of the galea and glossa in relation to the positioning of the corolla bend within the tube, also determine whether nectar can be reached (Table 3; Figs. 2–5). Before landing, bees swing their proboscids forward (the galea hinge does not bend beyond linear alignment with the bee's body). After landing on the lower lip the insect must angle its proboscis upwards into the corolla tube to access nectar at the base of the declined flower and since the proboscis is locked in linear alignment, the insect is forced to lower its body to raise the head and proboscis upwards. This action results in forced contact between the ventral surface of the insect's body and the anthers and style that are concealed in the lower lip, which facilitates pollen transfer. The lower lip does not return to its original position, and the anthers or stigma thus remain exposed for future visits.

Bees are covered with setae (hairs), especially along the groove between the legs on the ventral surface; these hairs generally point towards the posterior of the insect (Fig. 7g). As an insect arrives at the flower and lands on the lower lip (or contacts the sexual organs) the bifid stigma of a female phase flower dislodges pollen from between the hairs of the sternum onto the stigmatic surface. Pollen can only be picked up by the stigma as the insect moves into the flower and not as it retreats. Upon retreat from a male phase flower, pollen is passively loaded onto the hairs of the sternum, since the dehisced anthers brush close to the insect and force pollen to lodge between the hairs.

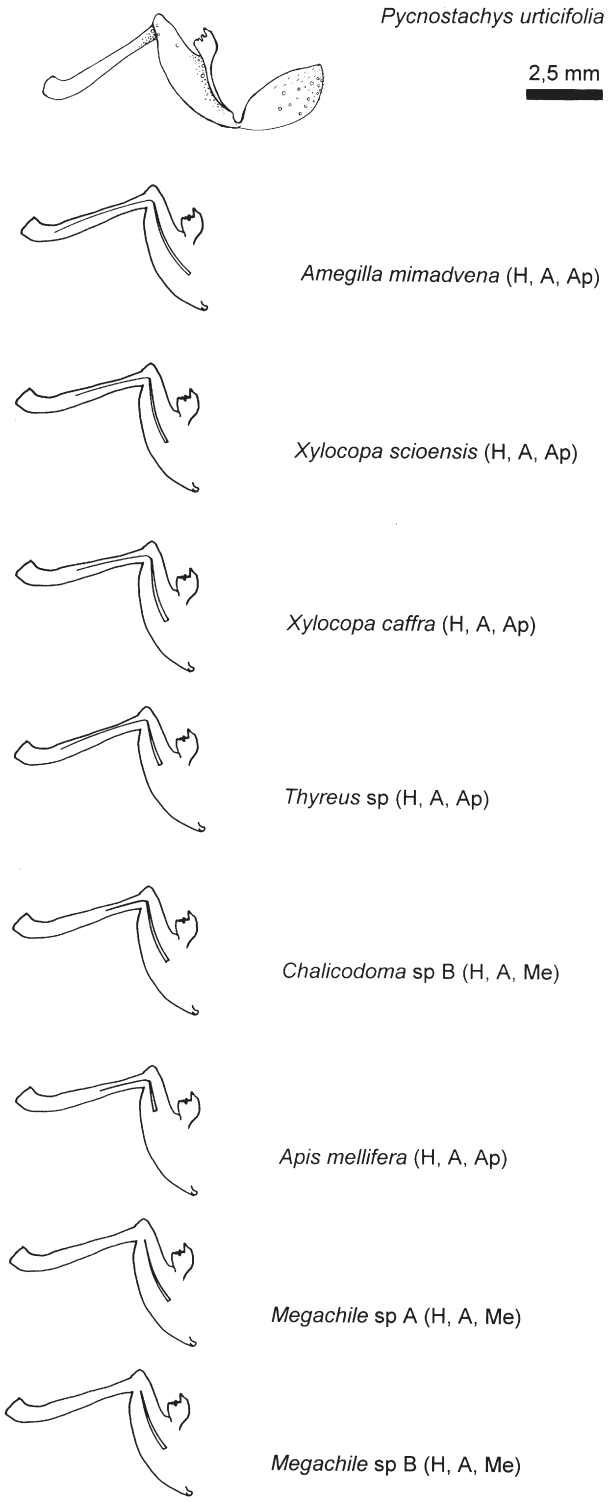

Fig. 5. Comparisons of floral tube shape and length with proboscids of insect visitors to *Pycnostachys urticifolia*. Single lines show flexible parts of proboscis, double lines show solid parts. H = Hymenoptera, A = Apidae, Ap = Apinae, Me = Megachilinae. Bar: 2.5 mm.

In all cases the pollinators of sigmoid species picked up and transported pollen on the ventral parts of their bodies (Table 4), with the thoracic area between the leg bases and the hairy area below the head being good sites for pollen carryover (Fig. 7g–h).

In the case of *P. calycinus* studied at Umtamvuna NR, no vouchers of *Xylocopa caffra* were caught, hence areas of pollen deposition could not be checked. Since bees crawl over the anthers and filaments to access nectar at the base of the floral tube, pollen placement is not always localised in discrete areas on the insect body. All visitors carried substantial amounts of *Plectranthus* pollen on their bodies, but the percentages of foreign pollen varied from 0 to 75% (Table 4).

Observations during the course of this study show that nemestrinid flies visit flowers for nectar; apinid bees visit flowers for nectar and sporadically for pollen collection, while megachilinid bees utilise nectar and/or load pollen onto the ventral abdominal scopae. With thoracic lengths of 13–18 mm and widths of 6–7 mm, apinid bees and nemestrinid flies comprise a class of large-bodied, nectar-feeding pollinators sufficiently powerful to depress the lower lip of a *Plectranthus* flower and pick up pollen from the anthers, but too broad to fully enter the mouth of a corolla tube (Table 5). Likewise, the smaller pollen-collecting megachilinid bees are also too large to fully enter into the laterally compressed corolla tubes (Table 5).

Results from the 2003 study on bee and fly visitor effectiveness on *P. laxiflorus* did not show a definitive increase or decrease as a result of fly emergence: average fruit set was 57.6% (SD = 19.5) before fly emergence (i.e. bees only), and 49.2% (SD = 22.8) after fly emergence (i.e. bees and flies). In seven inflorescences the fruit set was higher after fly emergence while in nine cases fruit set was lower. At a site in the Dargle it was noted that on an overcast morning flies were the only visitors to *P. laxiflorus* flowers, until about 11.30 am, when the sun came out and a few bees emerged. In this instance, *P. umbrosa* was the main floral visitor for the morning.

A few butterfly and one day-flying hawkmoth species feed on nectar of sigmoid *Plectranthus* species (Appendix 2: Lepidoptera); no pollen was found on the examined voucher specimens.

The pollination of three species with variously sigmoid corolla shapes was studied in less detail and is summarized as follows.

Plectranthus spicatus is a savanna species (Fig. 1d) with a succulent perennial habit, producing many decumbent stems from the base, with sub-spicate inflorescences that ascend up to 60 cm (Codd, 1985). The small flowers are blueish-purple in colour, with a sigmoid tube that is basally narrow, ascending at first and then curving sharply downwards, expanding at the throat (Figs. 6, 7d). Floral shape is similar to that of *Py. urticifolia*, but the corolla tube is shorter. *Xylocopa caffra* bees (proboscis length 6.5 mm) are the main pollinators of *P. spicatus* flowers at Oribi Gorge NR, with sporadic visits made by *Amegilla mimadvena* bees (proboscis length 9 mm).

Table 5
Range of body (thorax) size of insects that form the major pollinator groups of all seven studied plant species.

Insect group	Length of thorax (mm)	Width of thorax (mm)
Amegilla spp. (Apinid bee)	14–15	6–6.5
Xylocopa spp. (Apinid bee)	15–18	6–8
Prosoeca spp. (Nemestrinid fly)	13	7
Chalicodoma spp. (Megachilinid bee)	13–15	5–6

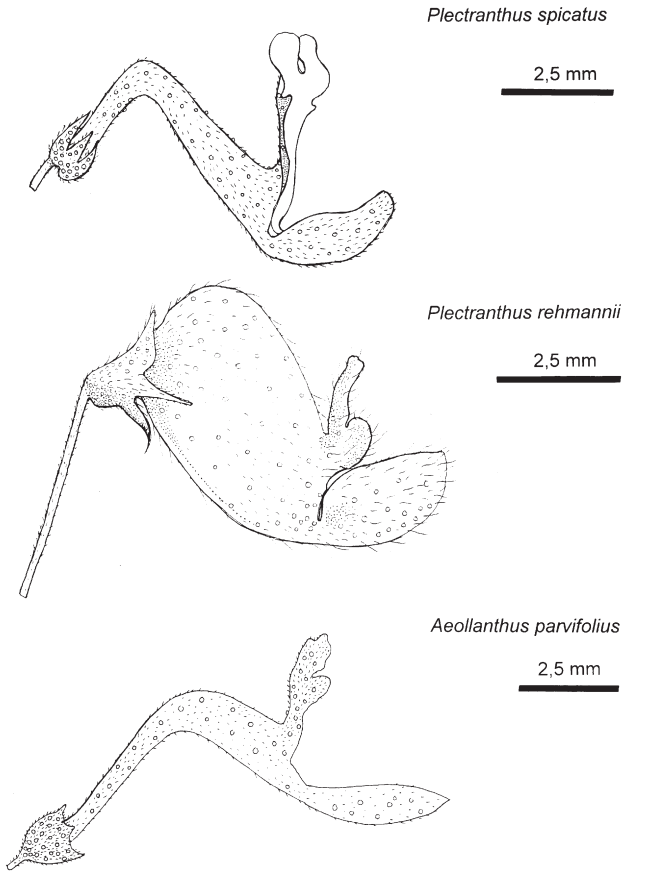

Fig. 6. Corollas of three additional *Plectranthus* and allied species studied: *Plectranthus spicatus*, *P. rehmanii*, *Aeollanthus parvifolius*. Bar: 2.5 mm.

Plectranthus rehmannii is endemic to the KZN Midlands (Fig. 1d), where it grows along or near forest margins. It is an erect, perennial sub-shrub reaching 1.2 m, with paniculate inflorescences up to 350 mm tall (Codd, 1985). The small flowers are creamy-white, with saccate bases and deflexed tubes (Fig. 6), similar to that of *P. calycinus*. At Leopard's Bush NR in the Karkloof, where *P. rehmannii* and *P. laxiflorus* grow in close proximity, the pollinator is a megachilinid bee, *Chalicodoma* sp. A (proboscis length 6 mm), which visits both species of *Plectranthus*. In a forest in the Dargle these two species also grow together, but none of the abundant *Prosoeca* flies at that site were seen to visit *P. rehmannii*. Honey bees (*Apis mellifera*) and *Allodape pernix* visited flowers of *P. rehmannii*, but only collected pollen from the anthers.

Aeollanthus parvifolius is a semi-succulent, perennial, herbaceous species that grows amongst rocks in grassland. The white to pinkish red flowers are borne on relatively lax, branched inflorescences. Cylindrical corolla tubes are 7–10 mm long, narrow at the base, expanding slightly towards the mouth (Codd, 1985), with a midway curve approaching the sigmoid shape. On the granite outcrops at Ongoye Forest (Fig. 1d), *Aeollanthus parvifolius* was pollinated by the apinid bees *Amegilla bothai*, *Amegilla mimadvena* and *Amegilla fallax*, all with proboscids ranging from 7 to 9 mm long. An acrocerid fly species (*Psilodera* sp.), with a flexible proboscis of similar length to those of the visiting bees, also probed flowers, but was not caught.

Estimations of proportional visitation by fly and bee pollinators to straight-tubed *Plectranthus* species showed that species that grow in or near shaded forest habitat tend to have more fly pollination visits, while species growing out in the open or in more sunny areas tend to have more bee visits (Table 6). This pattern is not clear in the sigmoid species, where most species are associated with sunlit patches in or near forest, or with sunny areas away from forest altogether.

5. Discussion

The observed modes of visitation by bees to sigmoid labiates generally correspond to that described by Scott Elliot (1891) and Stirton (1977). The nemestrinid flies (*Pr. umbrosa*, *Pr. circumdata* and *Prosoeca* sp. nov. 5, with proboscids 9–10.5 mm long) that visit *P. laxiflorus* and *P. calycinus*, are a new group of pollinators of sigmoid *Plectranthus* species. Nemestrinid flies with proboscids ranging from 8 to 30 mm also pollinate other, straight-tubed species of *Plectranthus* (Potgieter et al., 1999; Potgieter and Edwards, 2005).

There is an apparent close fit between the length of corolla tubes and the length of the proboscids of most apinid bees and nemestrinid flies that pollinate *P. laxiflorus* and *P. petiolaris* (as evidenced by visual comparisons in Figs. 2, 3). The flexible tips of bee proboscids accommodate the bend of the corolla tube, as does the flexibility of the nemestrinid proboscis along its whole length (Figs. 2, 3). These insects pick up pollen ventrally on the head, thorax and abdomen as the insect moves over the anthers. Species such as *Apis mellifera* (Fig. 2, *P. laxiflorus*) and *Allodape pernix* (Fig. 3, *P. petiolaris*) cannot reach nectar and rather collect pollen.

In the case of *P. calycinus* this fit by visual comparison only seems to hold for the apinid bee, *Xylocopa scioensis* (Fig. 4). Yet, despite its longer proboscis, the nemestrinid fly, *Prosoeca umbrosa*, picks up *P. calycinus* pollen on the hairy junction at the base of the head, as well as on the ventral surface of the thorax and abdomen (Table 4; Fig. 7h), when it appears as though only the base of the head should remove pollen from the anthers. The saccate base and inflated corolla of *P. calycinus* may be responsible for better contact between the anthers (and stigma) and the fly body, since the flexible proboscis may follow the curve of the upper part of the dorso-laterally flattened tube as it probes, which bends the proboscis and brings the fly body closer. Despite the weakly sigmoid shape of the corolla (it only bends close to the base) this design may function in excluding insects that do not have flexible proboscids in a way similar to other sigmoid corollas, while allowing for shorter-proboscid bees with flexible proboscids to probe directly towards the nectary by aligning with the lower part of the corolla (Fig. 4).

In *Py. urticifolia* the lengths and point of flexibility of proboscids of *Amegilla mimadvena*, *Xylocopa scioensis*, *X. flavorufa* and *Thyreus* sp. fit the corolla bend perfectly for nectar extraction and pollen removal (following visual comparison in Fig. 5). The recorded megachilinid bees cannot reach the corolla base for nectar. These bees actively collect pollen ventrally into abdominal scopae, in the same way as described by Percival (1965).

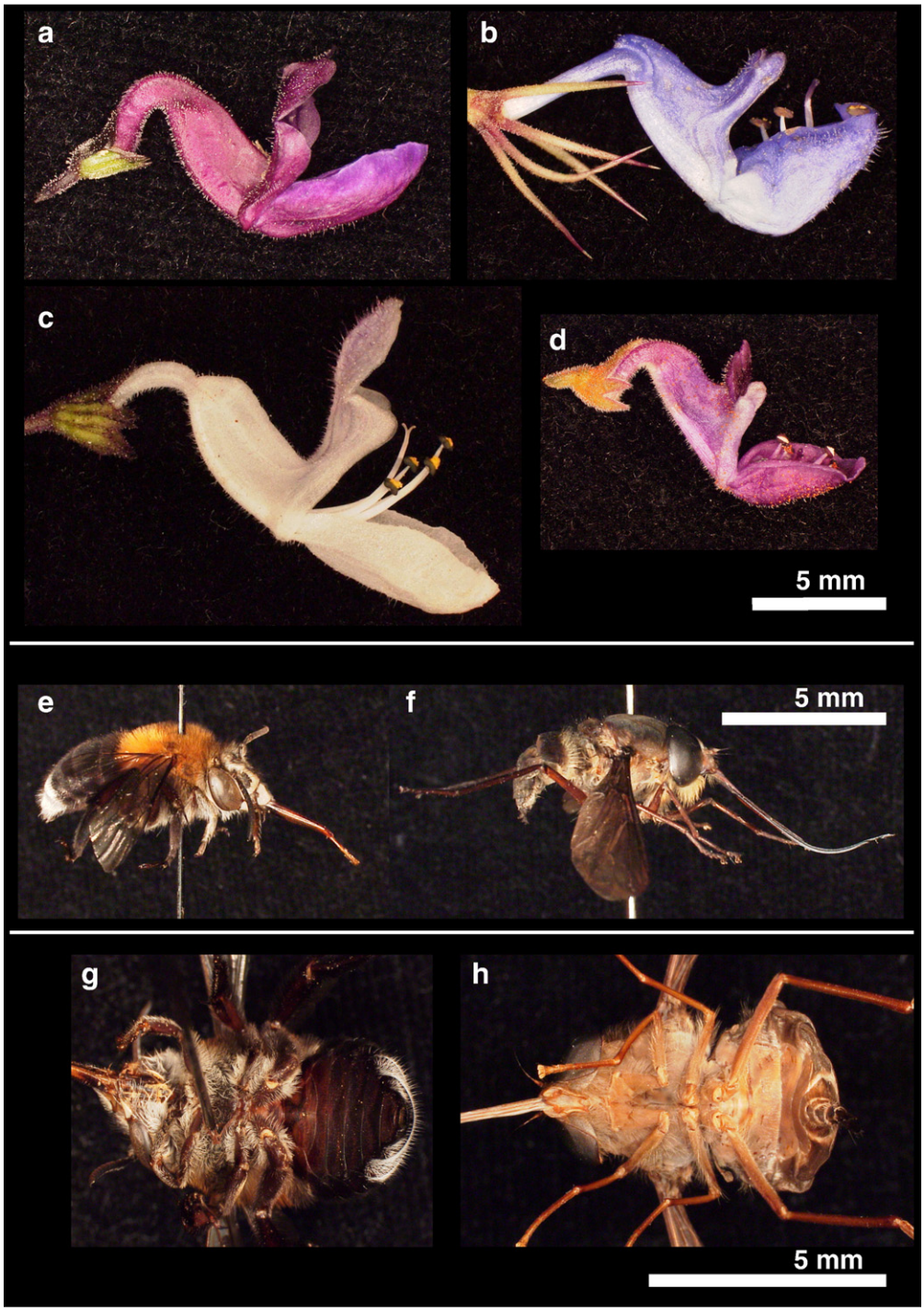

Fig. 7. A comparison of corollas of four sigmoid species (a–d), two insect pollinators (e–f) and undersides of an apinid bee and a nemestrinid fly (g–h). (a) *Plectranthus petiolaris*; (b) *Pycnostachys urticifolia*; (c) *Plectranthus laxiflorus*; (d) *Plectranthus spicatus*; (e) *Amegilla mimadvena* (Apinae, Hymenoptera); (f) *Prosoeca umbrosa* (Nemestrinidae, Diptera); (g) ventral surface of a typical apinid bee, *Amegilla mimadvena*, showing hairs on thoracic area and below the head; (h) ventral surface of a typical nemestrinid fly, *Stenobasipteron wiedemanni*, showing hairs below the head and groove between leg bases.

In general, the knee-like bend in the corolla tubes of *P. laxiflorus*, *P. petiolaris* and *Py. urticifolia* acts as a physical barrier in the corolla tube that only allows insect groups with flexible proboscids of sufficient length access to the nectar; the boat-shaped lower lip also limits the type of insect that can pick up pollen on its body. In *P. calycinus* this bend is less restrictive, sitting quite close to the corolla base, yet the two recorded visitors belong to bee and fly genera that also visit other sigmoid species. The same situation exists in *P. rehmanii*, while *A. parvifolius* has more gently curved corollas (Fig. 6). These act in much the same way as the knee-like bends discussed earlier, and that of *P. spicatus*, since a flexible proboscis is required to access nectar resources. The declined angle of the corolla tube forces insects into close contact with the reproductive parts of the

flower, but this means that an ascending tube may be necessary at the base of the corolla as it may prevent the gravitational bleed of nectar—the combination of these two evolutionary forces are most likely responsible for the sigmoid shape of the corollas. In a study on the pollination of *Disa versicolor* (Orchidaceae) it was found that there was a good fit between orchid spur length and that of bee mouthparts, while the sharply decurved spur of this species functions to accommodate the 'long curved tongue of *Amegilla* or similar large long-tongued bees' (Johnson, 1995).

The effect of the sigmoid corolla shape on forcing contact between insect and floral reproductive parts (i.e. anthers and stigma) is more pronounced in bees than in flies. The flight of the approaching, probing bee is interrupted by the need to settle on the lower lip and/or reproductive parts of the flower, since the proboscis locks in linear alignment with the insect's body; to accommodate the angle in the sigmoid corolla the insect body is forced below the horizontal. By settling there is close contact between the ventral surface of the bee body and the anthers/stigma and any movement by the bee to probe into or retreat out of the corolla tube leads to further chances of pollen deposition onto the bee and removal from the anthers. In nemestrinid flies the proboscis is more flexible and can bend to some extent to accommodate the sigmoid shape of the corolla tube, hence flies can hover while feeding and often bypass the anthers/style in the process. This happens during some visits, but observations also showed that the flies may grip the floral reproductive parts as they settle on the lower lip during feeding.

The short proboscis of *Allodape pernix* cannot legitimately reach the nectar in *P. petiolaris* (Fig. 3), other than by observed instances of robbing, where the bee pierces holes into the corolla base. This is akin to the robbing mentioned by Scott Elliot (1891) for *P. calycinus*, where a small 'fly' [sic.] was implicated. Species of *Plectranthus* with straight corolla tubes are robbed in a similar way by *A. pernix* (Potgieter et al., 1999), where the bees act as "primary nectar robbers" and possibly as secondary robbers as well, where nectar is accessed through holes made by primary robbers (Inouye, 1980).

Honey bees, *Apis mellifera*, are only able to reach the nectar in *P. laxiflorus* and *Py. urticifolia* (Figs. 2, 5) if the bees force their way into the corolla and if nectar levels are very high (e.g. at the start of the day). In most cases honey bees were observed rather to collect pollen from the anthers of flowers, as in the case of *Allodape pernix*. Observations in *P. petiolaris* show that while nectar may be more accessible to shorter-proboscid insects early in the morning, residual nectar is available later in the day only to longer-proboscid insects, when the bend in the corolla tube functions as an effective exclusion mechanism.

The short proboscids of megachilinid bees prohibit nectar feeding altogether, although these species collect copious amounts of pollen on the modified hairs (abdominal scopae) on the ventral surfaces of their abdomens. Unlike the situation in other bee families where the insects groom pollen off the body into scopae located on the hind legs (and hence out of the reach of stigmatic surfaces), the abdominal scopae of megachilinid bees make pollen constantly available for subsequent deposition on stigmatic surfaces. While this ensures effective pollination, it may lead to increased levels of geitonogamous fruit set, compared to other bees where pollen is sporadically groomed out of the reach of stigmas.

Although Van der Pijl (1972) suggested that sigmoid corollas may provide a shift from butterfly to bee pollination, the current study records both bee and a few butterfly visitors, thus the corolla shape allows access to both groups. Lepidoptera are, however, not effective pollinators of *Plectranthus* since they tend to bypass the reproductive parts of the flowers by hovering or briefly settling on the lower lip of the flower without contacting stigma or anthers.

The sigmoid species that have both bee and fly pollinators, such as *P. laxiflorus* and *P. calycinus*, pose interesting questions with respect to which pollinator group is more efficient at pollen carryover. The experiment conducted at Ferncliff NR to test this in *P. laxiflorus* does not, however, answer these questions fully. The results show little or no increase in fruit set later in the season, despite a considerable increase in pollinator activity when the nemestrinid flies emerge and forage actively alongside apinid bees.

Table 6
Twenty species of *Plectranthus* with straight or sigmoid, long or shorter corolla tubes listed according to habitat, showing proportions of bee and fly visits (C. Potgieter, unpubl. data).

Species of *Plectranthus* (or allied genus)	Habitat	% Flies	% Bees
Long-tubed, straight (20–32 mm)			
P. saccatus Benth. (long-tubed variety)	FOR: deep shade	100	
P. reflexus E.J. Van Jaarsveld and T.J.Edwards	FOR: deep shade	100	
P. hilliardiae Codd	FOR: deep shade	100	
P. ambiguus (H.Bol.) Codd	FOR: sunlit patches	80	20
Shorter-tubed, straight (4–18 mm)			
P. oertendahlii Th.Fries jun.	FOR: deep shade	100	
P. ciliatus E.Mey ex Benth.	FOR: deep shade and sunlit	60	40
P. zuluensis T. Cooke	FOR: shade	100	
P. praetermissus Codd	FOR: shade and sunlit patches	100	
P. fruticosus L'Hérit.	FOR: shade and sunlit patches/ margins	100	
P. ecklonii Benth.	FOR: sunlit patches/margins	70	30
P. oribiensis Codd	FOR/SUN: forest margins and wooded areas		100
P. madagascariensis (Pers.) Benth.	SUN: grassland, woodland, forest margin	50	50
P. ernstii Codd	SUN: sunny cliffs		100
Sigmoid-tubed (5–11 mm)			
P. petiolaris E.Mey. ex Benth.	FOR: sunlit patches/margins	1	99
P. laxiflorus Benth.	FOR: sunlit margins (late season)	50	50
P. rehmannii Gürke	FOR: shade and sunlit patches/ margins		100
Py. urticifolia Hook.	SUN: stream banks, sunlit forest margin		100
P. spicatus E.Mey. ex Benth.	SUN: grassland and dry woodland		100
P. calycinus Benth.	SUN: grassland	100	
A. parvifolius Benth.	SUN: sunlit rocky areas	25	75

FOR: species associated with forest habitat (sometimes on sunny forest margins); SUN: species in sunny habitats.% Flies represents an estimate of the number of visits received by effective fly pollinators in the families Nemestrinidae, Tabanidae and Acroceridae; % Bees represents an estimate of the number of visits received by effective bee pollinators in the apid sub-families Apinae and Megachilinae.

One explanation for this could be the genetic constraint on ovule number in the Lamiaceae: flowers of this family have just four ovules per flower, setting four nutlets per fruit. The sequential maturation of fruit did not allow for individual nutlets to be counted at the end of the season, since some of the smooth seeds were shaken from the calyces before the last fruits matured, hence the number of nutlets per fruit could not be considered a reliable count and only fruit set was represented here. The limited number of ovules in *Plectranthus* may explain why bee visitors alone effected as much fruit set as bees combined with flies, since only four pollen grains are needed to give full fertilisation of a fruit; any subsequent visits would not increase fruit set. Bees alone may then be adequate to provide maximum fruit set, or, at least, as much fruit set as when flies and bees are visiting together. While the maximal fruit set was not established with the aid of hand pollinations, it would seem likely that heavy visitation by two pollinator groups later in the season would account for most of the potential fruit set in the population.

The distribution of *P. laxiflorus* extends beyond that of the most abundant fly visitor, *Pr. umbrosa*, but where the plants do co-occur with the flies it is heavily visited by these flies (in addition to bees); in overcast conditions where bee activity may be limited, the flies may be the dominant visitor (as was seen at the Dargle site). This suggests that flies could be more important as pollinators in years with predominantly overcast weather. Most of the sigmoid species discussed here are limited to sunny or grassland habitats (*P. calycinus*, *P. spicatus*, *Py. urticifolia*, *A. parvifolius*), sunlit forest margins (*P. laxiflorus*, *P. rehmannii*) or open patches in forests and along forest margins (*P. petiolaris*) (Table 6). In other, straight-tubed species of *Plectranthus* there is a general pattern of species in shaded forest habitat being associated with a greater proportion of fly pollinators, while species in sunny areas (away from forest and forest margins) favour more bee pollinators (Table 6). This is confirmed by a study on pollination in a South African grassland community, where it was found that long-proboscid solitary bees were the most important floral visitors (Johnson et al., 2009-this issue). This pattern is not as clear in the sigmoid species, none of which occur in deep forest shade, but it could point to a trend in increasing reliance on fly pollinators adjacent to forests (e.g. *P. laxiflorus* with a 50:50 split in the latter part of the season—after fly emergence). The possibility exists that, as sigmoid species became more shade-tolerant and moved closer to forest habitat, they became pollen-limited as a result of bees not being active in denser forest shade; nemestrinid flies would then be of greater importance as pollinators. Nemestrinid flies are, however, also active in some grassland habitats (e.g. *P. calycinus*).

It is possible that nemestrinid flies are more efficient at pollen carryover than bees since the latter group of insects groom pollen off their bodies more frequently than flies do (S. Morita, pers. com.) At one stage it was thought that nemestrinid flies do not groom, but observations during this study confirmed that limited grooming happens during hovering and whilst resting. This happens especially to clean the eyes (S. Morita, pers. com.).

The observation that a species of acrocerid fly, *Psilodera* sp., visited *A. parvifolius* at Ongoye, where *Amegilla* bees were the more abundant floral visitors, is another case of a fly species occupying the same niche as a bee species. In this case the fly looks and sounds like one of the resident anthophorine bees, *A. fallax*. A similar observation on Iridaceae prompted Goldblatt et al. (1997) to describe a related acrocerid fly as a bee mimic. Acrocerid flies are pollinators of a number of straight-tubed *Plectranthus* species that are also pollinated by various species of *Amegilla* bees with similar proboscis lengths (Potgieter et al., 1999; Viljoen et al., 2006).

The phylogenetic work by Paton et al. (2004 and pers. com.) does not adequately explain the relationships between the different floral types of *Plectranthus*, hence the question of whether sigmoid tubes evolved from straight corollas, or vice versa, is difficult to answer. We suggest the possibility that straight corollas have evolved from sigmoid ones, for the following reasons.

Our data from *P. laxiflorus* shows that a shift from sigmoid to straight corolla tubes could occur since nemestrinid flies can accommodate the bend in sigmoid tubes. Why then did corollas straighten? When nemestrinid flies visit sigmoid corollas they do not always contact the anthers, since direct observations show that their flexible proboscids can bypass the anthers and stigma if the fly is hovering. This reduced efficiency is not significant where visiting insects are plentiful. However data collected from forest species of *Plectranthus* show widespread pollen limitation and field observations confirm that insect visits are rare (C. Potgieter, unpubl. data). Under these circumstances increased efficiency of pollen transfer would be strongly selected. By straightening the corolla tube the anthers and stigma are shifted into the flight path of flies which makes it more difficult to bypass. Those grassland labiate species (e.g. *Orthosiphon*, *Thorncroftia*) that are pollinated by nemestrinid flies all have straight corolla tubes (Potgieter and Edwards, 2001).

A shift from sigmoid to straight corolla tubes could explain the saccate corolla bases found in many straight-tubed forest *Plectranthus* species (e.g. *P. saccatus*, *P. reflexus*), since the saccate base may represent the upper remnants of a sigmoid corolla. In this study the corolla shapes of *P. calycinus* and *P. rehmannii* represent a possible intermediate situation of a saccate corolla base combined with a bend near the base. Only two forest *Plectranthus* species, *P. ambiguus* and *P. ecklonii*, have straight corolla bases. These questions will only be answered fully once a comprehensive phylogeny of *Plectranthus* and its allies, including all the long-tubed species from southern Africa, has been constructed.

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Appendix 1

Study site details and plant vouchers for species studied at each site.
KZN = KwaZulu-Natal Province; EC = Eastern Cape Province; NR = Nature Reserve.

Study site	Province	Quarter deg. grid	Plant species studied	Year studied	Voucher
Umtamvuna NR	KZN	3030CC	*P. petiolaris*	1995–1998	C. Potgieter 115
			P. calycinus	2000	
Oribi Gorge NR	KZN	3030CB	*P. petiolaris*	1996–1998	C. Potgieter 100
			P. laxiflorus	1998	
			P. spicatus	1998	C. Potgieter 142
Ferncliff NR: Pietermaritzburg	KZN	2930CB	*P. laxiflorus*	1996–2003	C. Potgieter 135
Leopard's Bush NR: Karkloof	KZN	2930CB	*P. laxiflorus*	1999	C. Potgieter 145
			P. rehmannii	1999	C. Potgieter 150
Dargle: KZN Midlands	KZN	2930AC	*P. laxiflorus*	2009	
			P. calycinus	2000	C. Potgieter 154
			P. rehmannii	2009	T. Edwards 3518
Ngeli Forest: Weza	KZN	3029DA	*P. laxiflorus*	1998, 2001	
Garden, Pietermaritzburg	KZN	2930CB	*Py. urticifolia*	1998	C. Potgieter 1064
Ongoye Forest: Empangeni	KZN	2831DC	*A. parvifolius*	1998	
Kologha Forest: Stutterheim	EC	3227CB	*P. laxiflorus*	1998, 2000	

Appendix 2

All observed insect visitors to flowers of the four species studied in detail: *Plectranthus petiolaris*, *P. laxiflorus*, *P. calycinus* and *Pycnostachys urticifolia*.

Localities indicated; U, Umtamvuna Nature Reserve; O, Oribi Gorge Nature Reserve; P, Pietermaritzburg (Ferncliff Nature Reserve); K, Karkloof (Leopards Bush Nature Reserve); D, Dargle area; N, Ngeli area; S, Stutterheim (Kologha forest).

P. petiolaris	*P. laxiflorus*	*Py. urticifolia*	*P. calycinus*
Hymenoptera	Hymenoptera	Hymenoptera	Hymenoptera
Apidae	Apidae	Apidae	Apidae
Apinae	Apinae	Apinae	Apinae
Amegilla mimadvena U, O	*Amegilla mimadvena* P, S	*Amegilla mimadvena* P	
Amegilla bothai O	*Amegilla bothai* P, K		
Amegilla caelestina U, O, P	*Amegilla caelestina* O	*Thyreus* sp. P	
	Apis mellifera P	*Apis mellifera* P	*Apis mellifera* D
Xylocopa hottentotta U	*Xylocopa flavicollis* S	*Xylocopa scioensis* P	*Xylocopa scioensis* U
Allodape pernix U	*Allodape ceratinoides* P	*Xylocopa flavorufa* P	
Halictinae	Halictinae		
Lasioglossum sp. O	*Zonalictus* sp. P		
	Megachilinae	Megachilinae	
	Chalicodoma sp. A K	*Chalicodoma* sp. B P	
		Pseudoanthidium truncatum P	
		Megachile sp. A P	
		Megachile sp. B P	
Diptera	Diptera		Diptera
	Nemestrinidae		Nemestrinidae
	Prosoeca umbrosa P		*Prosoeca umbrosa* D
	Prosoeca circumdata P, N, S		
	Prosoeca sp. nov. 5 P		
Syrphidae	Syrphidae		
Asarkina sp. O	*Asarkina* sp. A N		
	Asarkina sp. B K		
Episyrphus sp. U	*Oniromyia* sp. P		
	Voria sp. P		
	Bombyliidae P		
Lepidoptera	Lepidoptera	Lepidoptera	
Pieridae U	Pieridae S		
Lycaenidae U, O, P	Hesperidae K, P	Lycaenidae P	
	Papilionidae		
	Papilio nireus lyaeus S		
	Sphingidae		
	Macroglossum trochilus P, S		

References

Barraclough, D.A., 2006. An overview of the South African tangle-vein flies (Diptera: Nemestrinidae), with an annotated key to the genera and a checklist of species. Zootaxa 1277, 39–63.

Bezzi, M., 1924. The South African Nemestrinidae (Diptera) as represented in the South African Museum. Annals of the South African Museum 19, 164–190.

Brothers, D.J., 1999. Phylogeny and evolution of wasps, ants and bees (Hymenoptera, Chrysidoidea, Vespoidea and Apoidea). Zoologica Scripta 28, 233–249.

Codd, L.E., 1975. *Plectranthus* (Labiatae) and allied genera in southern Africa. Bothalia 11, 371–442.

Codd, L.E., 1985. *Plectranthus* (Lamiaceae). Flora of Southern Africa 28, 137–172.

Edwards, T.J., 2005. Two new *Plectranthus* species (Lamiaceae) and new distribution records from the Pondoland Centre of Plant Endemism, South Africa. Bothalia 35, 149–152.

Edwards, T.J., Paton, A., Crouch, N.R., 2000. A new species of *Plectranthus* from Zimbabwe. Kew Bulletin 55, 459–464.

Goldblatt, P., Manning, J.C., Bernhardt, P., 1997. Notes on the pollination of *Gladiolus brevifolius* (Iridaceae) by bees (Anthophoridae) and bee mimicking flies (*Psilodera*: Acroceridae). Journal of the Kansas Entomological Society 70, 297–304.

Huck, R., 1992. Overview of pollination biology in the Lamiaceae. In: Harley, R.M., Reynolds, T. (Eds.), Advances in Labiate Science. Royal Botanic Gardens Kew, Kew, pp. 167–181.

Inouye, D.W., 1980. The terminology of floral larceny. Ecology 61, 1251–1253.

Johnson, S.D., 1995. The pollination of *Disa versicolor* (Orchidaceae) by anthophorid bees in South Africa. Lindleyana 9, 209–212.

Johnson, S.D., Harris, L.F., Proches, S., 2009. Pollination and breeding systems of wildflowers in a southern African grassland community. South African Journal of Botany 75, 630–645 (this issue).

Lukhoba, C.W., Simmonds, M.S.J., Paton, A.J., 2006. *Plectranthus*: a review of ethnobotanical uses. Journal of Ethnopharmacology 103, 1–24.

Meeuse, A.D.J., 1992. Anthecology of the Labiatae: an armchair approach. In: Harley, R.M., Reynolds, T. (Eds.), Advances in Labiate Science. Royal Botanic Gardens Kew, Kew, pp. 167–181.

Paton, A.J., Springate, D., Suddee, S., Otieno, D., Grayer, R.J., Harley, M.M., Willis, F., Simmonds, M.S.J., Powel, M.P., Savolainen, V., 2004. Phylogeny and Evolution of Basils and Allies (Ocimeae, Labiatae) based on three Plastid DNA Regions. Molecular Phylogenetics and Evolution 31, 277–299.

Percival, M.S., 1965. Floral Biology. Pergamon Press, Oxford.

Potgieter, C.J., Edwards, T.J., 2001. The occurrence of long, narrow corolla tubes in southern African Lamiaceae. Systematics and Geography of Plants 71, 493–502.

Potgieter, C.J., Edwards, T.J., 2005. The *Stenobasipteron wiedemanni* (Diptera, Nemestrinidae) pollination guild in eastern southern Africa. Annals of the Missouri Botanical Garden 92, 254–267.

Potgieter, C.J., Edwards, T.J., Miller, R.M., Van Staden, J., 1999. Pollination of seven *Plectranthus* spp. (Lamiaceae) in southern Natal, South Africa. Plant Systematics and Evolution 218, 99–112.

Retief, E., 2000. Lamiaceae (Labiatae). In: Leistner, O.A. (Ed.), Seed Plants of Southern Africa: Strelitzia, vol. 10, pp. 323–334.

Scott Elliot, G.F., 1891. Notes on the fertilisation of South African and Madagascan flowering plants. Annals of Botany 5, 330–344.

Stirton, C.H., 1977. Broad-spectrum pollination of *Plectranthus neochilus*. Bothalia 12, 229–230.

Van der Pijl, L., 1972. Functional considerations and observations on the flowers of some Labiatae. Blumea 20, 93–104.

Van Jaarsveld, E.J., Edwards, T.J., 1991. *Plectranthus reflexus*. Flowering Plants of Africa 51, Plate 2034.

Van Jaarsveld, E.J., Edwards, T.J., 1997. Notes on *Plectranthus* (Lamiaceae) from southern Africa. Bothalia 27, 1–6.

Van Jaarsveld, E.J., Hankey, A., 1997. *Plectranthus venteri* Van Jaarsveld and Hankey spec. nov. (Lamiaceae), a new species from the Northern Province, South Africa. Aloe 34, 40–41.

Van Jaarsveld, E.J., Van Wyk, A.E., 2004. *Plectranthus mzimvubuensis*, a new species from Eastern Cape, South Africa. Bothalia 34, 30–32.

Viljoen, A.M., Demirci, B., Baser, K.H.C., Potgieter, C.J., Edwards, T.J., 2006. Microdistillation and essential oil chemistry — a useful tool for detecting hybridisation in *Plectranthus* (Lamiaceae). South African Journal of Botany 72, 99–104.

Vogel, S., 1954. Blütenbiologische Typen als Elemente der Sippengliederung. Botanischer Studien 1.

Winter, P.J.D., Van Jaarsveld, E.J., 2005. *Plectranthus porcatus*, a new species endemic to the Sekhukuneland centre of plant endemism, Limpopo Province, South Africa. Bothalia 35, 169–173.

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